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A STUDY OF NATURAL ANTIOXIDANT (VITAMIN E SYSTEM) IN MALIGNANT GROWTH

(Issledovaniye prirodnogo antioksidanta
[sistema vitamina E]
pri zlokachestvennom roste)

S. A. Burobina and Ye. A. Neyfakh

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A STUDY OF NATURAL ANTIOXIDANT (VITAMIN E SYSTEM) IN MALIGNANT GROWTH

by

S. A. Burobina and Ye. A. Neyfakh

In 1962 [2], on the basis of kinetic analysis of peroxide oxidation of endogenic unsaturated fatty acids (UFA) it was shown that in the process of development of a number of experimental tumors the main lipid antioxidant is redistributed. In connection with this we became interested in the vitamin E system, as it is one of the most important in preventing peroxide oxidation of endogenic essential fatty acids [6, 11, 13, 15]. The chief effect of vitamin E (tocopherol) as an antioxidant evidently consists of the neutralization of free radicals which develop in initial stages of spontaneous oxidation. Unfortunately, attempts to establish a connection between vitamin E and the process of malignant growth are limited to observations of tumor development [14] and do not concern the biochemistry of the metabolism of this important compound. The hypothesis that anemia accompanying malignant growth is connected with E avitaminosis has not been verified experimentally [10]. There are facts that α-tocopherol, unlike other fat-soluble vitamins, is absorbed by cancer cells at least as fast as by normal cells, but is lost by them more slowly if the tumor-bearing animal is transferred to a diet with a low content of vitamin E [16].

As vitamin E is not synthesized in animal tissues [7, 8] the theory has been expressed that tocopherol possibly migrates from tissues of the tumor-bearing animal to the growing tumor [11, 12]. To test this hypothesis we studied the vitamin E system (free tocopherol, esterified tocopherol and tocopherylquinone) in experimental malignant growth.

For analysis of the vitamin E system previously we suggested a comprehensive quantitative method [3] making it possible to analyze individual components of this system. This method consists of quantitative extraction of total lipids of animal tissues with subsequent identification of free (non-esterified) tocopherol (T_f), esterified tocopherol (T_e) and tocopherylquinone (T_q) using thin-layer chromatography and specific spectrophotometric determination. The advantages of this method are its high sensitivity and specificity.

The object of study in our experiments consisted of the following transplanted strains of malignant tumors: Walker's carcinoma of random bred rats, hematoma XXII of C_3HA mice, Ehrlich's ascitic carcinoma and ascitic sarcoma 37

of random-bred mice, as well as several tissues of tumor-bearing animals.

Ascitic forms of tumors were transplanted by means of injecting 5 million cells intra-abdominally, solid forms were transplanted subcutaneously. Controls were animals of the same strain, kept under identical conditions. To average results and smooth out individual differences we analyzed combined homogenates from the largest possible number of animals (3-7 per experimental point).

Quantitative study of tocopherol and its derivatives in tumorous tissues and tissues of tumor-bearing animals revealed a disturbance of vitamin E balance in malignant growth. In all tested tumorous strains a significant amount of tocopherol and its derivatives (T_e and T_q) was determined in the tumor itself with a simultaneous sharp reduction in the amount of vitamin E system in organs of the tumor-bearing animal. There were significant shifts in the content of individual components of the vitamin E system in tumorous tissues and organs of tumor-bearing animals.

CONTENT OF THE VITAMIN E SYSTEM IN MALIGNANT TUMORS AND LIVER OF TUMOR-BEARING ANIMALS

Object of study	Index, μg/g tissue			Liver*	Tumor
	T_f	T_e	T_q	$\frac{\Delta T_f}{\Delta T_e}$	$\frac{T_c}{T_e}$
Walker's carcinoma (14 days) . . .	37.9	1.2	8.1		
Liver. Test	1.7	2,8	9.5	3.75	3.16
Liver. Control	13.7	6.0	4.1		
Hepatoma XXII (11 days)	81.8	15.9	8.4		5.15
Liver. Test	7.4	7.1	18.2	0.67	
Liver. Control	21,1	27.5	58.8		
Cells of ascitic Ehrlich's carcinoma (11 days)	10.2	48.4	8.8		0.25
Ascitic plasma	2.1	1.6	1.0		
Liver. Test	14.3	0.03	8.8	0.28	
Liver. Control	21.4	25.7	18.9		
Cells of ascitic sarcoma 37 (11 days)	8.8	27.1	9.6		0.40
Ascitic plasma	2.8	1.8	1.1		
Liver. Test	5.2	0.8	6.5	0.65	
Liver. Control	21.4	25.7	18.9		

* ΔT — difference in content of tocopherol in control and test.

As seen from the table, combined tocopherol ($T_f + T_e$) is accumulated in tumorous tissues. At the same time the content of $T_f + T_e$ is reduced sharply in organs of tumor-bearing animals. The amount of free tocopherol in Walker's carcinoma is 96.9% of the total vitamin E content, in hepatoma XXII — 83.7%, in ascitic Ehrlich's carcinoma — 19.7% and in ascitic sarcoma 37 — 28.7%. The ratio of free and esterified tocopherol shows that 3.6 times more free tocopherol than esterified is accumulated in Walker's carcinoma and 5.15 times more in hepatoma XXII, while in ascitic Ehrlich's carcinoma free tocopherol comprises 0.25 of the esterified tocopherol content and in ascitic sarcoma 37 — 0.40.

Thus, vitamin E is represented in solid tumors primarily by free tocopherol and in ascitic strains basically by the esterified form.

The table shows that T_e decreases in the liver of animals with grafted ascitic tumors while in ascitic cells the concentration of this form of tocopherol is high. It is interesting that ascitic plasma contains significantly less $T_f + T_e$ than normal blood plasma [9]. Organs of animals with transplanted solid tumors in the majority of cases contain relatively little T_f, while in the tumor itself, on the contrary, vitamin E is represented basically in this form. The absolute content of tocopherol in the liver of animals with ascitic tumors is equivalent to its content in sarcoma 37 and comprises 50% of that in Ehrlich's carcinoma. The amount of combined vitamin E ($T_f + T_e$) in solid tumors is 2.9 times (Walker's carcinoma) and 5.6 times (XXII hepatoma) higher than in the liver of these tumor-bearing animals.

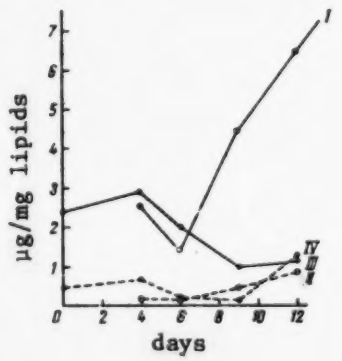

Figure 1. Redistribution of components of the vitamin E system between the liver and Walker's carcinoma and tumor-bearing rats

I — content of combined tocopherol ($T_f + T_e$) in Walker's carcinoma; II — tocopherylquinone (T_q) in the liver of tumor-bearing rats; III — combined tocopherol ($T_f + T_e$) in the liver of tumor-bearing rats; IV — tocopherylquinone (T_q) in Walker's carcinoma. Horizontally — time of development of tumors in days; vertically — content of components of vitamin E system in lipids, $\mu g/m^2$ of lipids.

The level of the oxidized form of tocopherol (T_q) in both the tumors themselves and in the liver of tumor-bearing animals is much lower than in the liver of normal animals. The content of free tocopherol in the liver of tumor-bearing animals varies from 12.2 to 70.1% of its content in the liver of normal animals. Esterified tocopherol in the liver of tumor-bearing animals comprises 1.50 to 47%. In other organs of tumor-bearing animals (kidneys, spleen) the tocopherol content is also reduced.

Quantitative changes in the vitamin E system are more significant in the malignant growth process (for example, in Walker's carcinoma). As the tumor grows the concentration of combined tocopherol in the liver of tumor-bearing animals is sharply reduced and at the same time is increased in Walker's carcinoma (Fig. 1). By the time of maximum development of Walker's carcinoma (9 days) the liver of the tumor-bearing animal loses about 60.2% of the combined tocopherol in comparison with its content in the liver of control animals. At the same time, 4.8 times more tocopherol is accumulated in the malignant tissue than in the liver of tumor-bearing animals. The level of T_q during the development of Walker's carcinoma gradually increases. In the interval between the 4th and 9th day of development of carcinoma the concentration of T_q drops 72.4% in the liver of tumor-bearing animals in comparison with that in intact animals, but the content of $T_f + T_e$ drops 3.6 times (Fig. 1).

As it has been established [1, 4, 11] that in tissues of Walker's carcinoma and in the liver of tumor-bearing rats total nonsaturation of endogenic fatty acids increases sharply, the increased concentration of T_q in these tissues must, evidently, be due to accelerated oxidation of $T_f + T_e$ in the process of preventing peroxide oxidation of these acids.

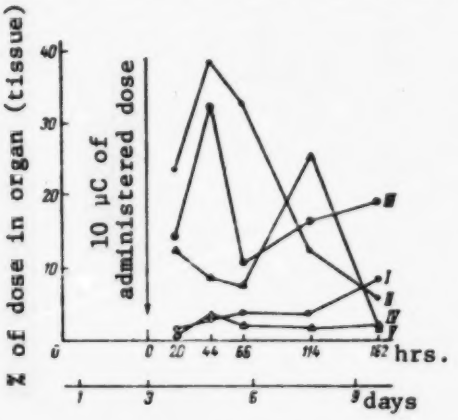

Figure 2. Percent of administered dose (10 μC) in liver, ascitic Ehrlich's carcinoma and blood of random-bred mice

I — ascitic Ehrlich's carcinoma, II — liver of tumor-bearing animals; III — liver of control animals; IV — blood of tumor-bearing animals; V — blood of control animals. Horizontally — time of development of tumor in days (lower scale) and time after administration of dose in hours (upper scale); vertically — % of dose in organ or tissue.

To reveal the mechanism of sharp shifts in the vitamin E system in malignant growth we performed experiments with radioactive tocopherol on two sharply differing strains of malignant tumors: ascitic Ehrlich's carcinoma and solid hepatoma XXII On the third day after inoculation with the tumor animals of the test and control groups were given 0.2 ml tail vein injections of a solution consisting of 20% blood plasma of animals of the same species, prepared in a physiological solution with 20 mg/ml Tween-80 and containing 10 μcurie dl-α-tocopherol — (5M-H^3).

Figure 2 presents curves reflecting the percent of inclusion of the administered dose (10 μcurie) in the liver, ascites and blood of tumor-bearing animals in comparison with the control. It is evident that by 20 hours after

the administration of labeled tocopherol, the inclusion of the label in the liver is almost 50% higher than in the liver of control animals; inclusion in the blood is also dissimilar in test and control groups of animals. At the maximum rate of development of the ascitic tumor (7-8th day) the level of included label is reduced in the liver and heart of tumor-bearing animals and sharply increased in kidneys, spleen and especially in the blood.

In ascitic cells inclusion rises quite slowly; from 66 to 114 hours after injection, inclusion of labeled tocopherol stays at the same level and then increases sharply. It is found that the percent of the dose of the label included in ascitic cells, calculated for one animal, is greater than in the kidneys, heart and spleen of control animals and by 162 hours — even greater than in the liver of tumor-bearing animals.

Thus, rapid excretion of the label from the liver of tumor-bearing animals is accompanied by its sharp increase in the blood with simultaneous accumulation of radioactive tocopherol by malignant cells. These facts indicate the humoral migration of vitamin E to malignant tissue from organs of tumor-bearing animals.

By the fourth day of development of hepatoma XXII the radioactivity absorbed by the liver of the "host" is excreted much faster than from an intact animal. Radioactivity of the blood of the tumor-bearer is higher, but then drops. These signs are accompanied by intensive inclusion of the label into tissue of the hepatoma, but radioactive tocopherol is gradually excreted from it, as from other organs of test and control animals.

Thus, these data indicate the redistribution of endogenic vitamin E between organs of the tumor-bearing animal and this strain of malignant tumor.

In the solid strain of malignant tumor — hepatoma XXII — we studied the distribution of the label in endogenic tocopherol and its derivatives during the development of the tumor. For this purpose the method of thin-layer chromatography was used to identify free tocopherol, esterified tocopherol and the oxidized form — tocopherylquinone.

Figure 3 shows that radioactivity in fractions of the vitamin E system are distributed: $T_q >> T_f + T_e$.

Therefore, radioactivity of T_q determines the total radioactivity of the entire vitamin E system.

Differential analysis of individual components of the vitamin E system showed that basic radioactivity of the administered labeled tocopherol is found in the spot corresponding to the oxidized form of tocopherol — tocopherylquinone (Fig. 3). Results indicate high metabolic activity of the phenol form of vitamin E. With the accumulation of tocopherylquinone in lipids of hepatoma

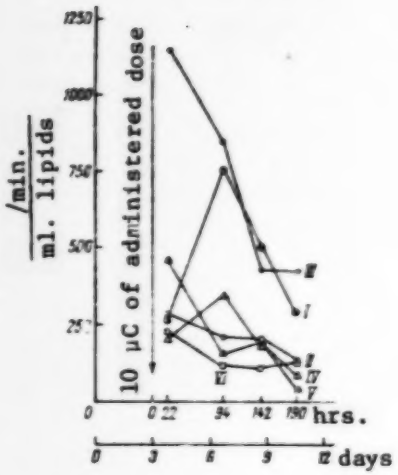

Figure 3. Distribution of radioactive components of the vitamin E system in the liver and hepatoma XXII of tumor-bearing mice.

I — tocopherylquinone (T_q) in hepatoma XXII; II — combined tocopherol ($T_f + T_e$) in hepatoma XXII; III — tocopherylquinone (T_q) in liver of tumor-bearing mice; IV — combined tocopherol ($T_f + T_e$) in the liver of control animals; V — tocopherylquinone (T_q) in the liver of control animals: VI — combined tocopherol ($T_f + T_e$) in the liver of tumor-bearing mice. Horizontally — time of development of the hepatoma in days (lower scale) and time after administration of the label (upper scale). Vertically — content of radioactive components of vitamin E in tumor and liver of tumor-bearing mice.

XXII it is excreted from the liver of test animals quickly, while the labeled fraction of combined tocopherol $T_f + T_e$ — slowly. It must be noted that any change in the level of the labeled phenol form of vitamin E in an intact liver is accompanied by an opposite change in the level of the labeled oxidized form of vitamin E, indicating their interconversion.

The difference in the distribution of the label in fractions of vitamin E from normal indicates the possible migration of vitamin E in the form of tocopherylquinone (T_q).

CONCLUSIONS

1. In the process of malignant growth organs of tumor-bearing animals lose endogenic tocopherol, simultaneously the corresponding form of tocopherol is accumulated in malignant tissues.

2. Vitamin E is accumulated in the malignant tumor, evidently, by means of its humoral migration from organs of the tumor-bearing animal.

3. Primary radioactivity of administered tocopherol is found in fractions of tocopherylquinone.

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